frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 24 January 2023
DOI 10.3389/fsufs.2022.1077274

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SPECIALTY SECTION
This article was submitted to
Sustainable Food Processing,
a section of the journal
Frontiers in Sustainable Food Systems

RECEIVED 22 October 2022
ACCEPTED 30 November 2022
PUBLISHED 24 January 2023

CITATION
Hoyos-Concha JL, Villada-Castillo HS, Roa-Acosta DF, Fernández-Quintero A and Ortega-Toro R (2023) Extrusion parameters and physical transformations of an extrudate for fish: Effect of the addition of hydrolyzed protein flour from by-products of *Oncorhynchus mykiss*. *Front. Sustain. Food Syst.* 6:1077274.
doi: 10.3389/fsufs.2022.1077274

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Extrusion parameters and physical transformations of an extrudate for fish: Effect of the addition of hydrolyzed protein flour from by-products of *Oncorhynchus mykiss*

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Introduction: The food industries play a fundamental role in feeding for the functions of animal metabolism. Fish feed extrusion cooking includes process-independent factors such as temperature (°C), screw speed (RPM), throughput, feed, and moisture content that influence the final product's nutritional value and physical properties. The evidence suggests that the application of hydrolyzed protein flour (HPH) is a crucial step for the techno-functional properties of the product. Therefore, this work aimed to study the effect of hydrolyzed protein meal from silage of trout (*Oncorhynchus mykiss*) on the parameters of the extrusion system and their physical transformations.

Methods: In this study, the influence of hydrolyzed protein meals ranges between 10 and 30% as a substitute for fish meals. The physical properties of the extrudate were monitored, evaluating the hardness, durability, buoyancy, expansion index, and apparent density.

Results: Consistent with this, parameters such as feed composition, screw speed, moisture content, and extrusion process affected the composition and properties of the final product.

Discussion: The physical properties indicated that the hydrolyzed protein flour presented cohesiveness and decreased the mean retention time in the extruder barrel and the specific mechanical energy (SME). Hydrolyzed protein flour during the extrusion process produces pellets with high durability and low hardness due to the high porosity presented, which allows for obtaining nutritional characteristics in the extruded product.

KEYWORDS

pellets, *Oncorhynchus mykiss*, cohesiveness, chemical silage, durability, specific mechanical energy, mean residence time

1. Introduction

Aquaculture (the farming of aquatic animals and plants) is a food activity that is dominating the world with a total production of more than 18.32 million tons (Tacon, 2020). Therefore, this sector requires quality food products for consumption with productive parameters. One of the critical sectors of aquaculture is the production of fish, such as trout, which requires considerable amounts of feed at a high cost. In this context, it is necessary to develop alternatives to replace commercial fish feed at least partially. One such alternative is fish silage. This type of fish food has certain physical properties, among which are hardness, durability, porosity, buoyancy, expansion rate, density, water absorption, and water solubility (Thomas and van der Poel, 1996; Cruz-Suárez et al., 2006; Chaabani et al., 2022). The physical quality tests of the diets must be conducted over some time, mainly if new ingredients, methodologies, or processing conditions are studied for the standardization of the product (Sørensen, 2012). In addition to nutritional aspects, fish feed has three main components within the pelleted feed, which are starch, lipids and protein of animal and/or vegetable origin, where their interaction influences the physical characteristics (Chen et al., 2010; Chaabani et al., 2022), such as porosity, good absorption, and a stable structure during storage, transport, and pneumatic feeding of the product (Aarseth, 2004; Aarseth et al., 2006; Draganovic et al., 2011).

Extrusion cooking of fish feed is a process that involves several variables and factors, both internal and external, to obtain the desired characteristics of the final product (Hoyos-Concha et al., 2022). During the process, independent parameters include temperature, process speed, throughput, feed composition, and feed moisture content. On the other hand, there are system variables also known as intermediate process variables such as specific mechanical energy and mean residence time that induces reactions that affect the nutritional value and physical properties of the final product (Choudhury and Gautam, 2003b; Choudhury et al., 2014). In this way, the composition of the feed plays a fundamental role in the variables of the process.

This study was conducted because, in the Department of Cauca (Colombia), there is high trout production, and several tons of waste are generated monthly. A strategy has been developed to take advantage of this waste and generate added value. Considering that fish feed has a high commercial cost, it is necessary to generate alternatives to reduce the price. In the present manuscript, an option to do so is proposed. The studies reported in this manuscript have served as the basis for scaling up the process at the pilot plant level. Therefore, this work aimed to study the effect of hydrolyzed protein meal from silage of trout (*Oncorhynchus mykiss*) on the parameters of the extrusion system and their physical transformations.

2. Methods

2.1. Materials

In the extrusion process, raw materials of hydrolyzed protein flour obtained from trout by chemical ensilage, fish flour (Siquality SA, Guayaquil-Ecuador), and cassava starch (sucre starch) were used. The proximate composition is presented in Table 1. Also, additives were used that were added to the diet such as vitamin core formulated for fish farming (Premex), Bentonite (Premex), calcium carbonate ($CaCO_3$; analytical degree 99%, Carlo Erba), and sodium chloride (NaCl; analytical grade 99%, Carlo Erba).

2.2. Materials and preparation of extruded product

The influence of hydrolyzed protein meal was investigated by using it as a replacement for fish meal, incorporating it in a range between 10 and 30%. The addition of supplements (vitamins, minerals, and starch) was based on the recommendations by the National Research Council (2011).

The diet formulation had a content of isoprotein and isoenergetic composition for omnivorous fish in the fattening stage. The flours were sieved with a No. 40 series Tyler sieve and blended in a Kitchen Aid mixer for 30 minu. They were later packed in polyethylene bags and refrigerated for 24 h, before being extruded. Raw materials required for the preparation of the diet are presented in Table 2.

2.3. Extrusion process of the feed

A compact single-screw extruder, Haake Polylab OS Rheomex 19/25 OS (Germany), was used, with a worm screw 475 mm long (L) and 19 mm in diameter (D) with a 25:1 L/D ratio. Their characteristics were a maximum temperature of 450°C, a total speed of 250 rpm and a maximum torque of 160 Nm, coupled to a Haake RheoDrive 4 system, with a motor power of 4 kW. The extruder barrel featured three independent heating or cooling zones; heating is conducted using electrical resistances and cooling through channels utilizing compressed air circulation. The compression ratio of the screw was 5:1, and the orifice diameter of the collet used was 5 mm. The equipment has three thermocouples to monitor the temperature of the barrel, an extra thermocouple placed in the matrix to measure the temperature of the product, and a device to measure the pressure. The feeding was done with the HAAKE Metering Feeder OS. The extruded samples were cut into pellets (length: 5 ± 0.1 mm) and dried in a BINDER brand dehumidifier (Germany) at 50°C until reaching a moisture content of 10 ±

TABLE 1 Means and standard deviations of total protein, ethereal extract, ash, fiber, free extract of nitrogen (ELN), total volatile nitrogen, pepsin digestibility, degree of hydrolysis, soluble protein, formic acid, and lactic acid of raw materials on a dry basis.

	Total protein (wt/wt %)	Ethereal extract (wt/wt %)	Ash (wt/wt %)	Fiber (wt/wt %)	ELN (wt/wt %)	Total volatile nitrogen (mg/100 g sample)	Pepsin digestibility (wt/wt %)	Degree of hydrolysis (wt/wt %)	Soluble protein wt/wt %)	Formic acid (wt/wt %)	Lactic acid (wt/wt %)
Hydrolyzed protein flour	62.92 ± 0.05	6.45 ± 0.02	17.86 ± 0.12	0.6 ± 0.2	12.2	109.4 ± 0.2	91.2 ± 0.2	62.948 ± 0.011	68.94 ± 0.09	0.41	0.18
Fish flour	63.20 ± 0.06	6.81 ± 0.02	18.67 ± 0.14	1.0 ± 0.2	10.3	109.70 ± 0.09	80.17 ± 0.006	5.61 ± 0.05	17.33 ± 0.06	0.19	0.18
Cassava starch	1.60 ± 0.05	0	0	2.16 ± 0.04	96.24	-	-	-	-	-	-

0.5%. Finally, the dry product was stored in polyethylene bags for properties analysis (Hoyos-Concha et al., 2022).

2.4. Evaluation of parameters of the extrusion system

2.4.1. Mean residence time (MRT)

Mean residence time (MRT) was measured with a marker based on previous research (Lee and Mccarthy, 1996). 0.05 g of erythrosine dye (Sigma Aldrich) was mixed with 5 g of starch adjusted to the moisture of the diet. The marker was added when the extruder reached steady-state conditions. Samples were taken every 10 s for 3 min after the addition of the title. The samples obtained were reduced in size using an IKA brand mill for fine grinding MF 10 basic (Germany) and subsequently sieved on a standard ASTM No. 50 sieve. The color of the pulverized material was determined on a Konica Minolta CR-400 colorimeter (USA) in triplicate. The intensity of the red color (C) was determined (Equation 1).

$$C = \sqrt{(a^*)^2 + (b^*)^2}$$

where a* represents the redness value and b* is the yellowness color value. The value of b* does not contribute to the intensity of the red color, therefore, the expression (Equation 2) is simplified.

$$C = a^*$$

The value of a * means a measure of the intensity of the red color in the extrudate (Bi et al., 2007). The mean residence time (TMR) or (t_m) is the mean residence time of a particle and was calculated according to what was proposed by Levenspiel (1999) (Equation 3).

$$m = \int_0^\infty E(t)\,dt = \frac{\sum_0^\infty tC\Delta t}{\sum_0^\infty C\Delta t}$$

where t_m represents the mean residence time in hours, C is the concentration of the marker at time t (h) and Δt (h) the time interval between samplings.

2.4.2. Specific mechanical energy (SME)

A sample was taken when the extruder reached a state of equilibrium, with constant torque and die pressure values, for ~5 min. For each treatment, the SME was determined by the following equation (Pitts et al., 2014) (Equation 4).

$$SME = \frac{n*\tau*P}{n_{max}*100*Q_m}$$

where n is the screw speed (rpm), P the power (kW), τ the torque (Nm), nmax the maximum screw speed (rpm) and Qm the total mass flow (kg/h).

TABLE 2 Raw materials required for the preparation of the diet.

Raw materials	Cassava starch	Hydrolyzed protein flour	Fish flour	Vitamin	Calcium carbonate	Bentonite	Sodium chloride
Incorporation (%)	55	10.0–30.0	30.0–10.0	2	0.8	1.7	0.5

2.5. Evaluation of physical changes of the extrudate

2.5.1. Porosity

The samples were obtained from cross-sections made with Ultramicrotome, LKB, Bromma (Sweden), adjusted to a cut of 30 μm, from the extruded pellets to determine the porosity. The internal diameters of the chambers formed in the pellet were captured by a Nikon DS-2Mv 2Mp digital camera (Japan), coupled to the Nikon eclipse 80i optical microscope (Japan), and processed in the Image Pro-Plus Analyzer Software (Version 6.3, 2008). The percentage of porosity obtained from the different treatments was determined.

2.5.2. Hardness

According to the procedure described by the ASAE Standard (1998), the test was conducted, which indicates the compression testing conditions of food materials with a convex shape. For this, measurements were made using a universal testing machine, Shimadzu EZ-L (Japan), with the following configuration: Test, Compression; Units, International System (SI); Load transducer, 500 N; Compression device, 13mm diameter solid steel cylinder; Moving head moving speed, 10 mm/min. The samples to be analyzed were conditioned in a Binder Model KBF 240 climatic chamber (Germany) at 50% relative humidity and 25°C, for seven days. Thirty-five representative samples were processed per treatment, 25 for the test and 10 for the moisture content measurement, which was homogeneous in their dimensions. The study processed the information in the Trapezium2 software.

2.5.3. Durability

A New Holmen NHP 100 (UK) Portable Durabilimeter equipment was used for the durability test. For this, a sample of pellets was taken and sieved through a No 16 Tyler series sieve, eliminating impurities and fine particles, to take 100 g finally. According to the pellet size, the test was conducted for 60 s, the pellets were collected, and the weight of the whole or whole material was obtained. The durability will correspond to the percentage of material that remained intact for the initial weight (Equation 5).

$$Durability\ index\ (\%) = \frac{Pf_{pellets}}{Pi_{pellets}} x\ 100$$

where $Pf_{pellets}$ is the final weight of the pellets (g), $Pi_{pellets}$ is the initial weight of the pellets (g).

2.5.4. Buoyancy

According to the methodology developed by Umar et al. (2013), it was conducted with modifications. In a 500 ml beaker, 400 ml of water were poured at 25°C, 100 pellets selected by quartering were taken, which were deposited in the water for 20 min. At the end of the time, the pellets that remained on the surface were quantified, and the percentage of buoyancy was determined (Equation 6).

$$Buoyancy\ index\ (\%) = \frac{Nf}{Nt} x\ 100$$

Where Nf is the number of pellets floating after 20 min, Nt is the total number of pellets in the test.

2.5.5. Expansion index (EI)

The expansion index of the extruded products was determined according to the methodology described by Draganovic et al. (2011). The diameter of 10 pellets obtained by quartering from each treatment was determined with a Mitutoyo Co (Japan) digital vernier caliper. The average value was obtained and divided by the extruder nozzle diameter (5 mm; Equation 7).

$$EI = \frac{D_p}{D_b}$$

where EI is the expansion index, Dp is the average diameter of the pellet (mm), and Db is the diameter of the nozzle (mm).

2.5.6. Apparent density

The apparent density of the extruded products was obtained assuming the cylindrical shape of each pellet, following the method proposed by Singh et al. (2012). Ten samples of pellets obtained by quartering of 5 mm in length were used, the mass was determined using a Kern AES 220-4 Analytical Balance (Germany). Height and diameter determination was performed using a Mitutoyo Co (Japan) digital vernier caliper. The bulk density of the samples was determined (Equation 8).

$$DE = \frac{m}{\pi * r^2 * l}$$

where DE is the density of the pellet (kg/m^3), m is the mass (kg), *r* is the radius (m), and *l* is the length (m).

2.6. Statistical analysis

Table 3 shows the 2^K factorial design to evaluate the parameters of temperature, screw speed, moisture content, and inclusion of hydrolyzed protein flour, with two levels each. The factorial experiment was extended by adding axial and central points to obtain a response surface design, with a main composite design of 31 treatments in total, distributed as follows: 16 treatments (1–16) corresponding to the factorial portion, eight treatments (17–24) corresponding to axial points and seven treatments (25–31) corresponding to central themes.

The axial points were obtained by applying the term $\alpha = (F)^{1/4}$, where F is the number of points used in the factorial portion of the design, where $\alpha = 2$. The values of the axial points were extrapolated according to the coding presented in Table 5. Minitab 16 software was used for the response surface analysis.

3. Results

3.1. Extrusion system parameters

Table 4 presents the summary of the results obtained from the central composite design for each of the response variables. Once the fulfillment of the normality assumptions had been verified, the analysis of variance was carried out using linear regression. The non-significant terms were eliminated according to F and P-values, considering a significance level of 0.05. With the ANOVA adjusted for each of the variables, the regression coefficients were obtained for the construction of the mathematical models, corresponding to each response surface as a function of mean retention time (TMR), specific mechanical energy (SME), index of expansion (EI), density, porosity, hardness, durability and buoyancy, these results are presented in Table 3. All the models suggested a lack of acceptable fit (P-value > 0.05), and according to the regression analysis, it can also be stated that the effects considered in Table 4 explain the variability in a percentage higher than 70%. They were obtained in the data. Furthermore, the existence of curvature in the experimental plane evaluated can be inferred for all response variables due to the presence of quadratic terms with significant effect (P-value < 0.05).

3.1.1. Specific mechanical energy (SME)

The F values estimated in the ANOVA (Table 4) indicate that the factors hydrolyzed protein flour (67.02) and moisture content (20.09), followed by the quadratic effects of temperature (27.10) and hydrolyzed protein flour (13.22), has the most significant influence on SME. In Table 5, it is verified that the regression coefficient of the screw speed has a positive effect on the SME (1.796). This fact means that the SME increased by increasing the screw speed. Although the impact of screw speed is low, some authors attribute the change to an increase in torque in the equipment's motor. Factors such as engine torque, die pressure, and SME were closely related. Besides, Figure 1 shows the effects of HPH, moisture content and temperature on SME.

According to Figure 1A, it can be verified that SME decreased when there were a high concentration of moisture and hydrolyzed protein. However, this decrease is much more pronounced because of the hydrolyzed protein compared to the impact that the change in moisture content in the mixture could generate. On the other hand, in Figure 1A, it is observed that as the concentration of hydrolyzed protein decreases, the SME increases, which can be explained by a higher concentration of fishmeal (high molecular weight protein). The effect of temperature and hydrolyzed protein can be seen in Figure 1B that hydrolyzed protein has a notable impact on the impact of weather on SME. As in the previous case, it can be verified that with the increase in the content of hydrolyzed protein flour, the SME decreased, independent of the evaluated temperature.

3.1.2. Retention mean time (TMR)

The F values estimated in the ANOVA (Table 4) show that the individual factors of hydrolyzed protein flour (161.82) and moisture (56.68) had a more significant influence on the TMR. Similarly, there was essential participation of the pure quadratic term of the screw speed (13.75) and the effect of hydrolyzed protein flour with moisture and screw speed. Figure 2 shows the impact of HPH, moisture content and screw speed on TMR.

3.2. Evaluation of the physical changes of the extrudate

3.2.1. Expansion index (EI)

The F values estimated in the ANOVA (Table 5) indicated that the hydrolyzed protein flour factors (153.9) and temperature (115.3) exerted a more substantial influence on the response variable. Furthermore, the only interaction with a significant effect was moisture and hydrolyzed protein flour (3.43).

According to Table 4, the EI ranged between 1.01 and 1.85. Figure 3A shows that the increase in hydrolyzed protein concentration provoked an increase in EI.

3.2.2. Density

Figure 4 shows the effect of HPH, moisture content and temperature on density. Figure 4A shows that the product's density decreased with the concentration of hydrolyzed protein flour. In Figure 4B, it is observed that the hydrolyzed protein flour had a more significant influence on the change in the density of the product obtained compared to the effect that the temperature change can exert. However, the interaction

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TABLE 3 Experimental design based on temperature, moisture, hydrolyzed protein flour (HPH) and screw speed (RMP).

Treatment	Temperature (°C)	Moisture (%)	HPH (%)	RPM (min^{-1})	SME (W.h/kg)	MRT (s)	EI	Density (g/cm^3)	Porosity (%)	Hardness (*N*)	Durability (%)	Buoyancy (%)
1	120	18	15	205	76.67	109.78	1.37	0.832	13.52	111.17	96.13	72.3
2	130	18	15	205	73.54	116.48	1.15	0.761	17.35	98.76	93.70	100.0
3	120	22	15	205	61.06	88.94	1.4	0.869	13.11	109.93	96.87	93.7
4	130	22	15	205	46.96	90.61	1.17	0.849	15.8	87.72	97.90	98.3
5	120	18	25	205	57.72	82.44	1.68	0.830	20.49	92.28	99.87	100.0
6	130	18	25	205	27.95	83.22	1.48	0.789	17.36	116.58	97.83	99.0
7	120	22	25	205	26.17	71.42	1.65	0.760	16.33	78.77	100.87	100.0
8	130	22	25	205	26.87	77.85	1.4	0.814	17.98	103.64	97.20	98.3
9	120	18	15	235	110.64	97.25	1.35	0.820	11.69	110.77	92.03	85.7
10	130	18	15	235	80.30	108.40	1.06	0.703	18.59	70.76	89.97	99.3
11	120	22	15	235	67.00	86.96	1.48	0.953	15.6	97.45	97.57	90.0
12	130	22	15	235	49.63	86.46	1.1	0.918	17.25	72.17	95.57	98.7
13	120	18	25	235	32.47	66.34	1.69	0.802	20.99	83.03	95.10	100.0
14	130	18	25	235	30.56	61.37	1.5	0.815	17.72	80.83	94.50	100.0
15	120	22	25	235	25.06	64.00	1.65	0.774	17.4	84.04	97.70	98.0
16	130	22	25	235	24.16	50.21	1.43	0.795	17.24	75.68	98.80	99.7
17	115	20	20	220	25.23	79.08	1.54	0.851	15.72	97.58	98.30	64.0
18	135	20	20	220	18.23	87.41	1.01	0.738	22.39	74.71	96.10	100.0
19	125	16	20	220	97.76	103.15	1.54	0.765	17.03	134.91	90.93	73.7
20	125	24	20	220	55.47	59.27	1.55	0.964	11.23	120.45	96.87	99.3
21	125	20	10	220	129.84	120.94	1.28	0.790	15.27	106.52	92.90	61.7
22	125	20	30	220	61.21	68.85	1.85	0.789	29.87	54.11	98.10	100.0
23	125	20	20	190	50.60	92.63	1.54	0.884	5.48	150.16	98.80	52.7
24	125	20	20	250	60.72	59.81	1.55	0.901	9.92	131.57	94.90	20.3
25	125	20	20	220	69.00	88.59	1.44	0.979	6.59	170.17	97.53	41.7
26	125	20	20	220	62.42	90.48	1.52	1.011	5.94	186.6	99.53	20.0
27	125	20	20	220	51.85	91.98	1.49	1.003	4.95	168.54	98.40	26.3
28	125	20	20	220	52.36	98.25	1.62	0.956	6.16	184.94	97.90	47.0
29	125	20	20	220	65.17	90.36	1.54	0.918	4.65	158.64	95.80	32.7
30	125	20	20	220	55.15	79.87	1.6	0.891	7.69	162.15	96.20	36.0
31	125	20	20	220	45.80	93.44	1.59	0.888	7.03	159.76	97.17	37.0

Results of specific mechanical energy (SME), mean residence time (MRT), expansion index (EI), density, porosity, hardness, durability and buoyancy of the obtained extruded feeds.
HPH, hydrolyzed protein flour; RPM, screw speed in revolutions per minute.

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TABLE 4 Regression analysis of specific mechanical energy (SME), mean residence time (MRT), expansion index (EI), density, porosity, hardness, durability, and buoyancy of the obtained extruded feeds.

Parameter	SME		MRT		EI		Density		Porosity		Hardness		Durability		Buoyancy	
	F	Coef.	*F*	Coef.	*F*	Coef.	*F*	Coef.	*F*	Coef.	*F*	Coef.	*F*	Coef.	*F*	Coef.
Constant	27.02	57.941	32.18	91.560	53.97	1.518	8.97	0.9495	30.81	0.061	24.24	170.11	21.90	97.433	11.25	34.38
Temperature	4.03	−4.617	0.86	1.006	115.3	−0.254	3.74	−0.035	6.32	0.019	2.75	−8.920	9.57	−0.628	2.90	10.47
Moisture	20.09	−10.313	56.68	−8.192	0.00	0.001	12.37	0.065	3.97	−0.016	1.68	−6.972	52.26	1.467	0.94	5.97
HPH	67.02	−18.838	161.8	−13.842	153.9	0.293	2.29	−0.027	30.72	0.043	5.31	−12.391	44.64	1.356	3.28	11.14
RPM	0.046	1.796	40.11	−6.891	0.00	−0.001	0.25	0.009	2.06	0.011	6.25	−13.441	30.59	−1.122	0.56	−4.58
Temperature × temperature	27.00	−10.862	3.75	−1.919	51.21	−0.306	25.18	−0.167	89.74	0.135	93.12	−95.074	–	–	32.09	63.86
Moisture × moisture	–	–	6.00	−2.428	–	–	8.43	−0.097	36.26	0.086	29.53	−53.593	19.51	−0.813	36.78	68.36
HPH × HPH	13.22	7.587	–	–	–	–	26.78	−0.172	142.4	0.170	104.88	−100.89	5.03	−0.413	30.93	62.69
RPM × RPM	–	–	13.75	−3.676	–	–	4.27	−0.069	2.30	0.022	16.77	−40.350	–	–	2.65	18.36
Temperature × moisture	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Temperature × HPH	–	–	–	–	–	–	–	–	6.86	−0.049	6.91	34.632	–	–	–	–
Temperature × RPM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Moisture × HPH	–	–	5.29	3.065	3.43	−0.1075	10.10	−0.1412	–	–	–	–	4.9	−0.550	–	–
Moisture × RPM	–	–	–	–	–	–	–	–	–	–	–	–	10.26	0.796	–	–
HPH × RPM	–	–	4.71	−2.893	–	–	–	–	–	–	–	–	–	–	–	–
Adjusted R^2	81.26	90.34	92.78	70.51	89.94	87.47	84.79	73.22								
Lack of adjustment (*P*-value)	0.081	0.616	0.813	0.759	0.05	0.346	0.901	0.069								

HPH, hydrolyzed protein flour; RMP, screw speed.

TABLE 5 2^4 factorial design with reserved levels.

Factors	Temperature (°C)		Screw speed (min⁻¹)		Moisture (%)		HPH (%)	
Levels	120	130	205	235	18	22	15	25
Coding	−1	1	−1	1	−1	1	−1	1

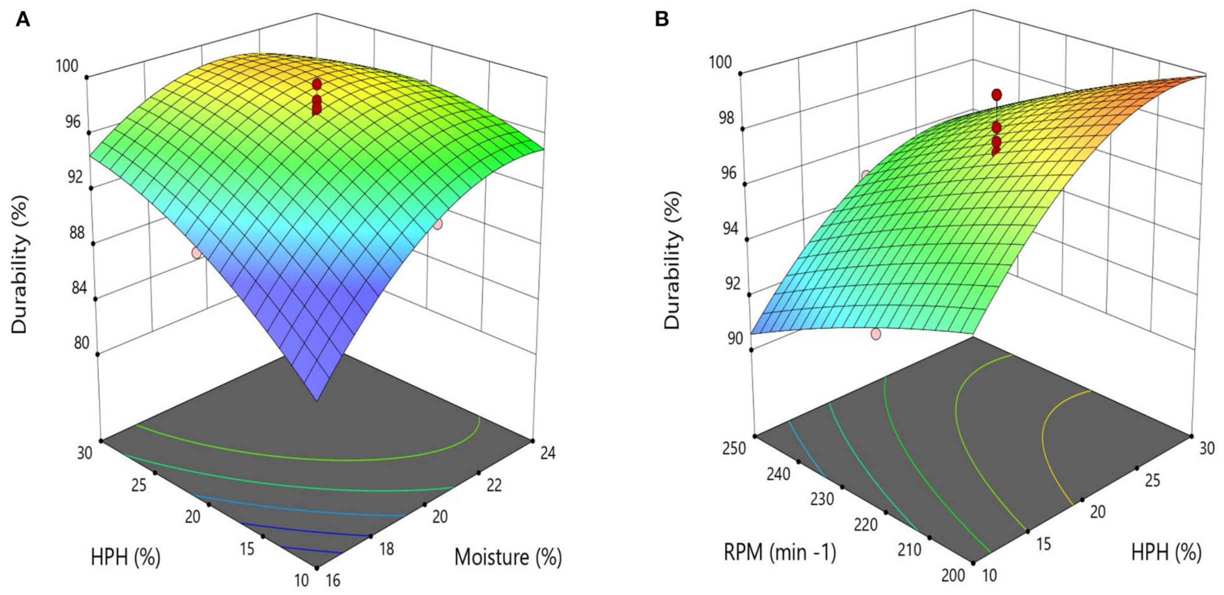

FIGURE 1
Effect of HPH, moisture and temperature on EME. **(A)** Moisture vs. HPH (temperature 125°C and screw speed 220 RPM). **(B)** HPH vs. temperature (screw speed 220 RPM and humidity 20%).

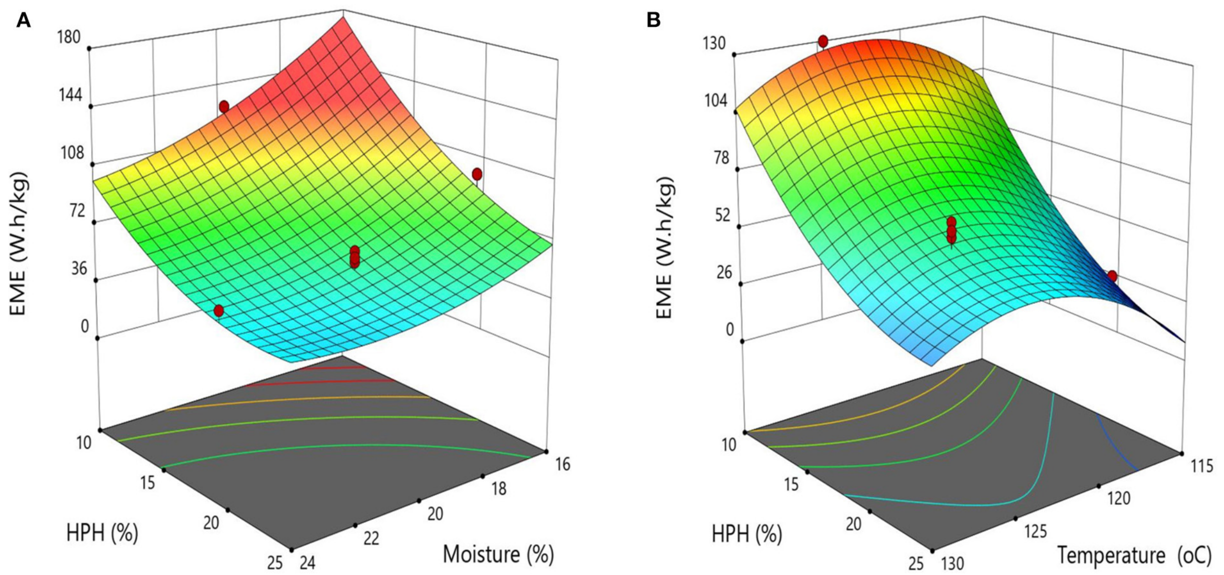

FIGURE 2
Effect of HPH, moisture and screw speed on TMR. **(A)** Moisture vs. HPH (temperature 125°C and screw speed 220 RPM). **(B)** Screw speed vs. HPH (moisture 20% and temperature 125°C).

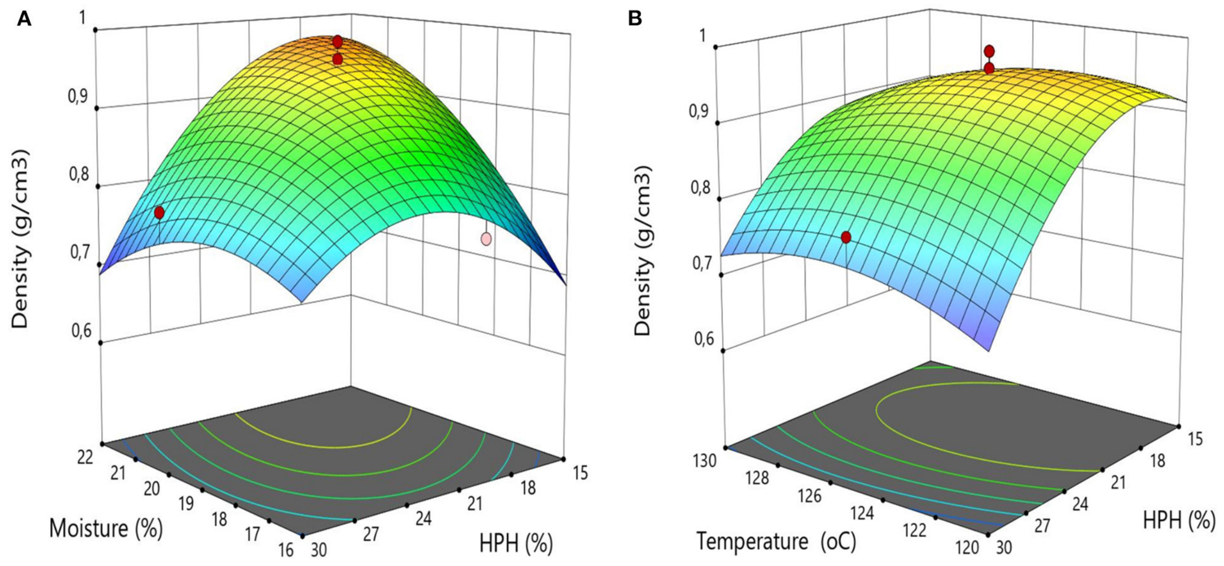

FIGURE 3
Effect of HPH, moisture and temperature on EI. **(A)** Moisture vs. HPH (temperature 125°c and screw speed 220 RPM). **(B)** HPH vs. temperature (screw speed 220 RPM and moisture 20%).

of temperature and hydrolyzed protein flour increased density when extrusion conditions converge on an intermediate temperature profile of 128°C and a concentration of hydrolyzed protein flour of 20%. On the other hand, the change in density attributable to screw speed is low. In Table 5, it is observed that the terms that represent the screw speed, independent ($F = 0.25$) and pure quadratic ($F = 4.27$), contributed little to the change in density.

3.2.3. Hardness

In Figure 5A, it is observed that there is a more significant effect of the concentration of hydrolyzed protein flour on hardness compared to the impact that a change in moisture content could exert during the extrusion process. It was also found that the lowest level of hardness of the extrudates was achieved when the content of hydrolyzed protein flour was at its maximum concentration (30%). Secondly, in Figure 5B, a more significant influence is corroborated by the variation of hydrolyzed protein flour in the mixture, compared to the impact generated by temperature. However, when the temperature in the extruder barrel was low, and the concentration of hydrolyzed protein flour was high (30%), the hardness of the material showed an apparent decrease. Table 5 shows the effect of screw speed and the regression coefficient for this parameter.

3.2.4. Durability

Table 5 shows the F values estimated in the ANOVA as moisture content (52.26) and hydrolyzed protein meal (44.64) as independent terms with more influence on durability. Figure 6A shows that the maximum durability of the pellets was obtained when the moisture content was between 20 and 24% and the concentration of hydrolyzed protein flour was kept between 25 and 30%. In Figure 6B, the high durability of the pellets can be observed when the screw speed was 190 rpm, while the content of hydrolyzed protein flour in the mixture was high (30%).

3.2.5. Buoyancy

The values of F estimated in the ANOVA (Table 5) indicate that the pure quadratic terms of temperature (32.09), moisture content (36.78) and hydrolyzed protein flour (30.93) were those that had the most significant influence on buoyancy. Figure 7 shows the effect of HPH, moisture content and temperature on buoyancy. According to Figure 7A, the maximum percentage of buoyancy of the pellets was reached when the extrusion process was carried out with 24% moisture content and 30% hydrolyzed protein flour. These observations agree with the minimum density (Figure 4A) achieved at said extrusion conditions.

4. Discussions

4.1. Extrusion system parameters

4.1.1. Specific mechanical energy (SME)

According to Figure 1A, it can be verified that SME decreased when there was a high concentration of moisture

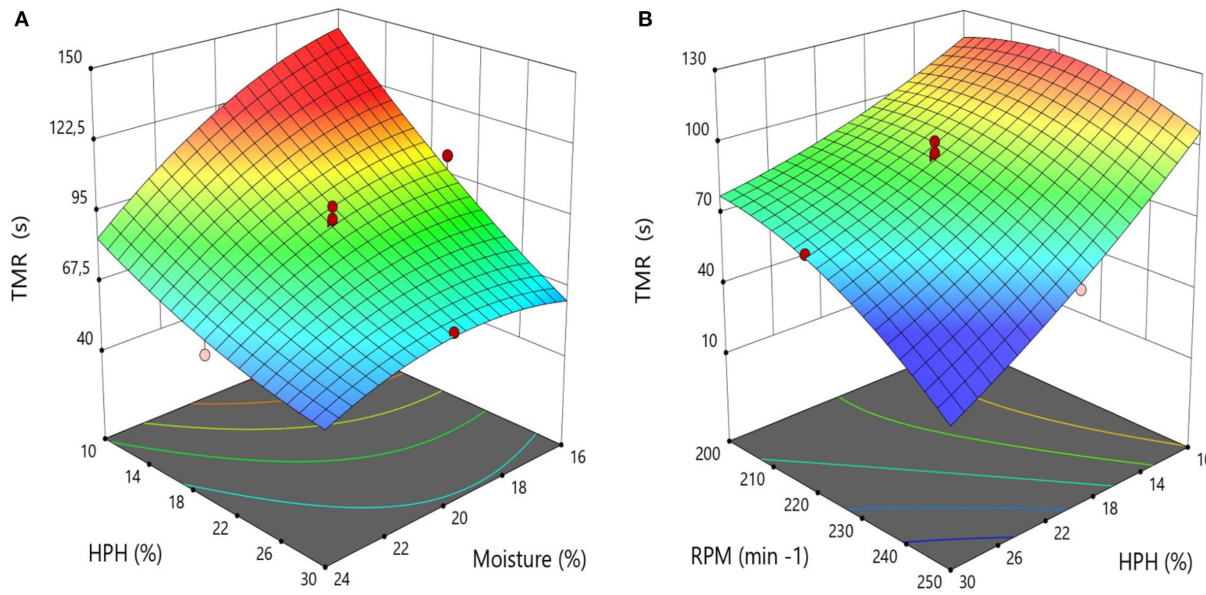

FIGURE 4
Effect of HPH, moisture and temperature on density. **(A)** Moisture vs. HPH (temperature 125°C and screw speed 220 RPM). **(B)** HPH vs. temperature (screw speed 220 RPM and moisture 20%).

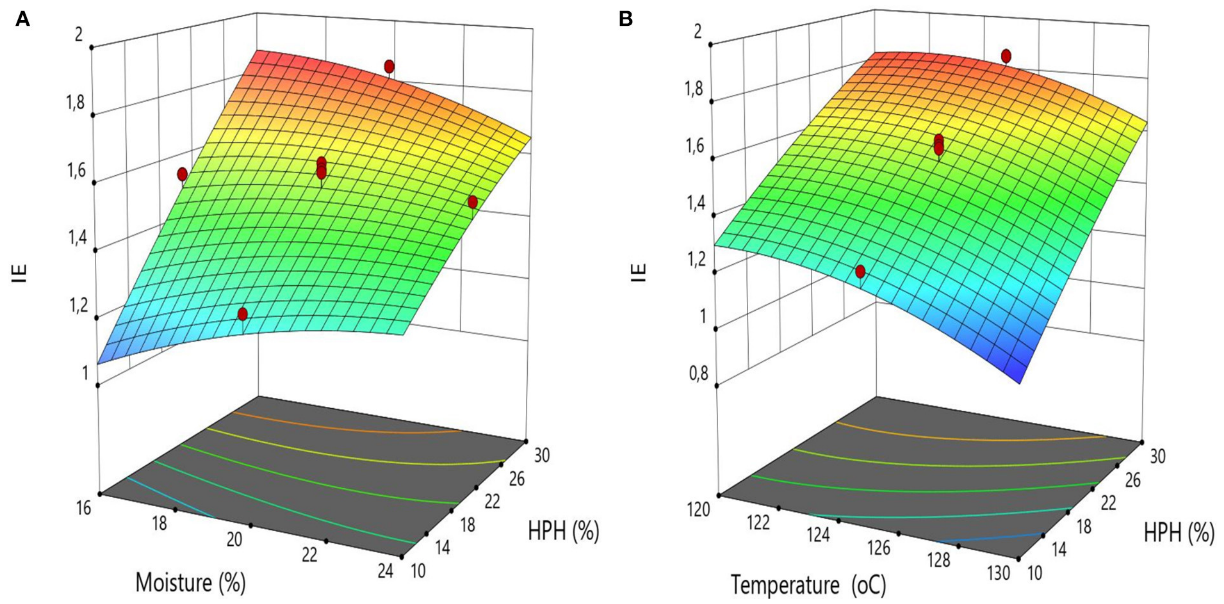

FIGURE 5
Effect of HPH, moisture and temperature on hardness. **(A)** Moisture vs. HPH (temperature 125°C and screw speed 220 RPM). **(B)** HPH vs. temperature (screw speed 220 RPM and moisture 20%).

and hydrolyzed protein simultaneously. However, this decrease was much more pronounced because of the hydrolyzed protein than the impact that the change in moisture content in the mixture could generate. On the other hand, in Figure 1A, it is observed that, as the concentration of hydrolyzed protein decreased, the SME increased; this behavior could be attributed to a higher concentration of fish meal (high molecular weight protein). The effect of temperature and hydrolyzed protein can be seen in Figure 1B that hydrolyzed protein had a notable impact on the impact of weather on SME. As in the previous

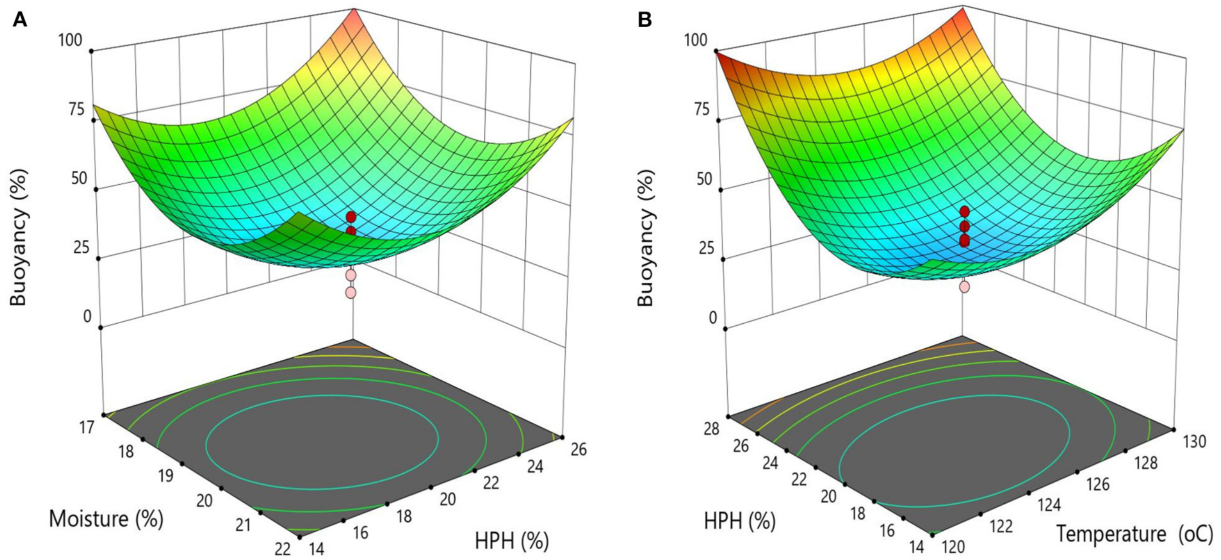

FIGURE 6
Effect of HPH, moisture and screw speed on durability. **(A)** HPH vs. Moisture (temperature 125°C and screw speed 220 RPM). **(B)** HPH vs. screw speed (temperature 125°C and moisture 20%).

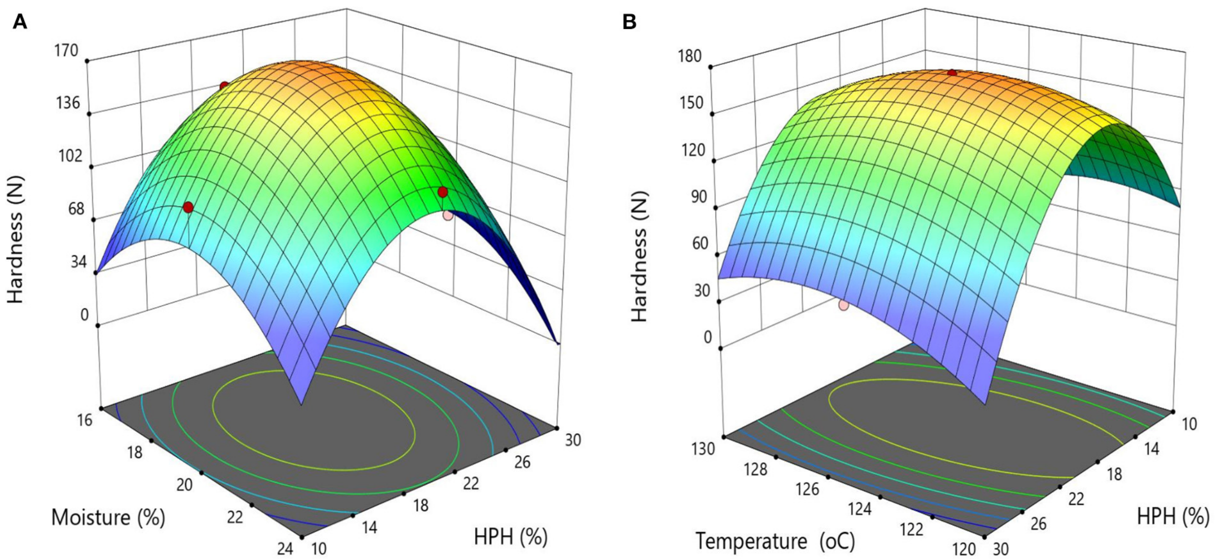

FIGURE 7
Effect of HPH, moisture and temperature on buoyancy. **(A)** Moisture vs. HPH (temperature 125°C and screw speed 220 RPM). **(B)** HPH vs. temperature (screw speed 220 RPM and moisture 20%).

case, it can be verified that with the increase in the content of hydrolyzed protein flour, the SME decreased, independent of the evaluated temperature. The decrease in SME at low or elevated temperatures was probably due to reduced viscosity in the mixture fed to the extruder (Singha et al., 2018). At the same time, they were related to the level of transformation of the product obtained and its characteristics such as expansion, density, geometric and texture characteristics (Pansawat et al., 2008).

Similar studies reported that the SME applied to a mixture in specific parameters was affected by the complex viscosity of the mixture during the fusion process and the mixture by the torque needed to turn the screws. Therefore, a mixture with higher viscosity and a lower processing temperature needs a higher torque and, by extension, a higher SME than mixtures in conditions where the viscosity was lower (Ma et al., 2019; Thompson and Williams, 2021).

The use of low temperatures avoided the gelatinization of the starch and its interaction with the protein. In addition, the plasticizing effect of the proteins allowed the mobility and fluidity of the mixture through the extruder (Samuelsen and Oterhals, 2016). On the other hand, elevated temperatures also decreased SME, but the behavior was probably due to the rapid dextrinization of carbohydrates and the breakdown of previously formed protein complexes. This effect also facilitated the movement of the melted mixture through the extruder (Day and Swanson, 2013; Miranda et al., 2014). The increase in SME at an intermediate temperature condition could be explained by the rise in the viscosity of the molten material (Pansawat et al., 2008).

4.1.2. Retention mean time (TMR)

Regarding Figure 2A, it can verify that the TMR had a more significant change when the concentration of hydrolyzed protein flour decreased, keeping the moisture content low. The concentration of hydrolyzed protein and the indirect effect of the concentration of fish meal present in the mixture could explain this behavior. A low concentration of hydrolyzed protein (low molecular weight protein) was related to a higher concentration of fishmeal (high molecular weight protein), which can absorb and retain water more strongly and limits the efficient distribution of moisture available, resulting in a decrease in plasticizer in the mix and addition in TMR (Jean et al., 2005; Singh et al., 2012; Samuelsen et al., 2014). By increasing the concentration of hydrolyzed protein and the mixture's moisture content, the TMR's lowest value was recorded. In this case, the protein and the moisture content function as plasticizers facilitating the mobility of the mixture in the extruder. However, excess moisture and a high level of hydrolysis of fish protein decreased viscosity so much that it was difficult for the material to exit the extruder (Choudhury and Gautam, 2003a). In Figure 2B, it was observed that the high screw speeds decreased the TMR, according to that reported by Yu et al. (2014), presenting a more efficient heat dissipation, greater pumping force, and more significant shear, giving way to better mixing of the material and a short TMR (Kumar et al., 2006). A drop in TMR was observed at extreme temperatures and slightly increased when the process was at intermediate temperatures. The fall of the TMR may be a function of a loss of the mixture's viscosity inside the extruder, facilitating the transit of the material through the barrel. Said thickness loss occurred when low molecular weight protein concentration and its plasticizing effect increased (Samuelsen et al., 2014) at the low-temperature point. On the other hand, increasing the temperature to the maximum level also generated a drop in the viscosity of the molten material, requiring less effort to exit the extruder (Kumar et al., 2006).

4.2. Evaluation of the physical changes of the extrudate

4.2.1. Expansion index (EI)

This behavior could be associated with the low molecular weight of the hydrolyzed protein. Although this type of protein allowed a uniform moisture distribution, it also facilitated the gelatinization of starches in the hydrothermal process (Samuelsen et al., 2014). At the same time, these proteins provided sufficient material for interaction between components, facilitating a nucleation effect for the formation of cells, regulating the release of water vapor, and maintaining the expanded structure at the exit of the extruder (Singh and Muthukumarappan, 2016).

Figure 3B shows the change in EI due to the change in hydrolyzed protein and temperature concentration, finding that the effect of the concentration of hydrolyzed protein flour was more significant (Table 5). This figure verifies that maintaining a concentration of hydrolyzed protein flour equal to 30% and conducting the extrusion at temperatures below 120°C were sufficient conditions to achieve a higher EI. This fact was probably because the temperatures were sufficient to achieve gelatinization of the available starch (Dileep et al., 2009) and facilitate the interaction between proteins-carbohydrates through interactions by hydrogen bonds, ionic bonds, and hydrophobic interactions (Samuelsen and Oterhals, 2016), generating stable networks or structures at the exit of the extruder, facilitating an increase in EI. On the other hand, if the temperature was increased beyond 120°C, it contributed to a decrease in the viscosity of the molten material, the product of the excessive degradation of the components in the mixture, causing low stability of the networks formed at the exit from the extruder and deficiencies in the filling of the barrel, all these mechanisms generated a consequent decrease in EI (Pansawat et al., 2008; Valenzuela et al., 2017).

On the other hand, it has been reported that a low screw speed reduces shear forces, generates less pressure inside the barrel in the extruder and decreases radial expansion (Majumdar and Singh, 2014). Excess screw speed produces a structural collapse of the extruded food material, reducing the EI (Valenzuela et al., 2017).

4.2.2. Density

The apparent density (g/cm^3) quantifies the mass of the material per unit volume of the extrudates. It measures the extrudates' internal structure (Kannadhason et al., 2010). The molten mixture suffers expansion as it exits the extruder. According to Figure 4A, the density of the product decreased with the concentration of hydrolyzed protein flour, which made the moisture present in the mixture easily distributed, favoring the gelatinization of the starch, interactions with protein and the nucleation effect with a increase in pressure (Dileep et al., 2009).

When making a comparison of the results of EI and density, it can be stated that achieving a very low density does not necessarily imply a maximum expansion, as observed in Figures 3A, 4A, where the highest level of increase was completed at the point hydrolyzed protein flour 30% and moisture content 16%. Density and EI were negatively correlated in any extrusion process, behavior related to the distribution of cells inside the extrudate, size, and thickness of the walls that they form makeup (Zarzycki et al., 2015).

The lowest level of density was achieved at a low screw speed and a maximum concentration of hydrolyzed protein flour in the mixture, indicating that a low screw speed was sufficient to facilitate a kneading of the mix that allowed the interaction between the low molecular weight proteins and available carbohydrates, promoted the shear effect, the pressure inside the extruder and the distribution of moisture content (Cheftel et al., 2014).

4.2.3. Hardness

The hardness is considered a property that allows information about the physical quality of the pellets (Samuelsen and Oterhals, 2016) and is positively correlated with increased density and durability (Majumdar and Singh, 2014). The behavior of Figure 5 could be attributed to a higher concentration of low molecular weight protein, which favored a higher level of starch gelatinization, with the generation of expanded, porous, and low-density structures (Ozer et al., 2004), characteristics that were inversely correlated to hardness.

Zhang et al. (2021) reported that at low humidity levels, the hardness of the product decreased as the cooking temperature increased. In addition, as the extrudate emerged from the cooling nozzle, there was a tendency to swell slightly, weakening the structure and leading to lower hardness.

4.2.4. Durability

The durability of an extrudate is related to the ability of the solid material to maintain a complete structure when subjected to external mechanical stress, such as wear due to fragmentation and abrasion (Kaliyan and Vance, 2009; Samuelsen et al., 2013). Figure 6 shows the effect of HPH, moisture content, and screw speed on durability.

According to Figure 6A, the maximum durability of the pellets was obtained when the moisture content was between 20 and 24%, and the concentration of hydrolyzed protein flour was kept between 25 and 30%. These combinations could be constituted in an adequate ratio of components for adequate mixing of the molten material, homogeneity, and increasing durability (Samuelsen et al., 2014). The decrease in durability in the pellets occurred when the process was carried out at low levels of hydrolyzed protein flour (10%) and low moisture contents (16%), due to the high molecular weight of fishmeal, which had a great influence on the result, since it prevented the starch from achieving an effective gelatinization process, avoiding the interaction of components during the fusion of the mixture (Samuelsen et al., 2013; Ah-Hen et al., 2014).

In the Figure 6B, this behavior could be explained by the effectiveness of the shear generated under these extrusion conditions, which may be sufficient to allow the components in the mixture to achieve their melting temperature, with the consequent formation of a durable structure (Samuelsen et al., 2014).

Also, the durability of the pellets reached its lowest level when the screw speed was increased (250 rpm), and the concentration of hydrolyzed protein flour in the mixture was decreased (10%). This behavior was influenced by the high concentration of high molecular weight proteins (heavy flour) in the protein fraction of the mixture, limiting the availability of moisture, which means that the starches were unable to gelatinize, as explained above. In addition, it prevented fusion, shear, and coupling of low molecular weight proteins, causing a decrease in the durability of the material (Jobling et al., 2007).

Ishak et al. (2022) studied different starch sources influencing the physical parameters of extruded foods prepared for the Malaysian mahseer, *Tor tambroides*. Forty percent protein and 23.4% starch were used in the methodology, and they were extruded using a single screw extruder (120 rpm speed, 2 mm diameter die head). Among the results, it was found that the starches from three local crops (sago, *Metroxylon sagu*; manioc, *Manihot esculenta*; and taro, *Colocasia esculenta*) produced extrudates with a quality like the extrudates of corn starch in terms of expansion ratio, durability index, durability index, and buoyancy index. However, electron microscopy indicated cassava starch extrudates had the best external appearance.

By verifying the effect of temperature on its regression coefficient (Table 5), its negative effect concerning the durability of the pellet can be established. This behavior could be attributed to the fact that the temperature profiles close to 115°C were sufficient to ensure that the molten mixture that passes through the extruder could induce proteins to quickly join with the rest of the components in the mixture, improving the durability of the pellets (Kaliyan and Vance, 2009).

On the other hand, if the temperature profiles used in the process were high (135°C). At the same time, the content of hydrolyzed protein flour was low (10%), extrudates of a non-cohesive nature were generated and, therefore, pellets of low durability (Jobling et al., 2007; Chevanan et al., 2008).

4.2.5. Buoyancy

Buoyancy is the number of floating pellets suspended in the aqueous medium, observed for some time. Furthermore, the buoyancy of the pellets has been inversely associated with density, stability to water, higher absorption, and low solubility. In this sense, the buoyancy of the pellets is guaranteed if the density is below 750 kg/m^3. Authors such as de Cruz et al. (2015) stated that the pellets float if the thickness was below 530 kg/m^3, while authors such as Mjoun and Rosentrater (2011) stated that there was a guaranteed buoyancy if the pellets have a density lower than 1 g/ml. These variations could be a function of the dimensions of the pellets and their composition (Vassallo et al., 2005).

Regarding the effect of the speed of the screw and according to its regression coefficient of the model, it has a significant quadratic effect on the change in buoyancy (2.65). However, its impact was positive and of low influence on others. Variables considered buoyancy increased at the extreme levels of this parameter and decreased at intermediate values. This behavior was that reported with the density analysis (Table 5), where its quadratic effect, apart from being significant, its influence was negative, being inversely correlated variables (Chevanan et al., 2008; Draganovic et al., 2011; Mjoun and Rosentrater, 2011; Ah-Hen et al., 2014; de Cruz et al., 2015).

5. Conclusions

Regarding the cohesion capacity (crosslink), the physical properties of the granules, such as buoyancy, expansion index, porosity, hardness, and durability, were studied. Hydrolyzed protein flour had cohesive and plasticizing capacity. The plasticizing capacity generated a reduction in the average retention time in the extruder cylinder and a decrease in the specific mechanical energy. Although HPH had a cohesive action, the increase in mass flow due to the plasticizing effect impacts the SME, reducing its value. Durability was the response variable that showed a positive correlation. In the study, high-durability pellets were achieved due to the cohesiveness of the hydrolyzed protein flour, but at the same, time low hardness due to the high porosity achieved. Therefore, porosity was one of the response variables in which the addition of hydrolyzed protein flour had a higher incidence. The fish feed obtained in this work has a high potential to replace, at least partially, commercial fishmeal, having a positive impact on production costs. It has also served as the basis for scaling production to a semi-industrial scale in the Department of Cauca, Colombia.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Comité de ética de la Universidad del Cauca.

Author contributions

Conceptualization, formal analysis, funding acquisition, investigation, and methodology: JH-C. Data curation: JH-C and DR-A. Project administration: JH-C and HV-C. Resources and validation: JH-C, HV-C, and AF-Q. Software and writing—original draft: JH-C and RO-T. Supervision: HV-C and AF-Q. Visualization: JH-C and AF-Q. Writing—review and editing: JH-C, DR-A, and RO-T. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors thank Universidad del Cauca and the financial support of the General System of Royalties through project ID 3883.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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